EFFECT OF X-IRRADIATION (POST LARVATION) ON DEVELOPMENT, MORTALITY AND ANTIGENICITY OF *Ascaridia galli* (Schrank, 1788)

by

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INTRODUCTION

In recent years there has been a rapid increase in the use of ionizing radiation in many fields of physical and biological science. An increasing amount of research has been performed aimed at the elucidation of the effects of ionizing radiation on biological systems, including parasites and their hosts. Two major areas are the development of ionizing irradiation techniques useful in the public health for destroying trichina in pork, and the production of "living antigens" of helminths by X-irradiation. Along with such practical and economically important uses of radiation, much basic research must be done to expand knowledge of irradiation effects.

The objectives of this study were; (1) to extend the work of Babero (1952) to X-irradiation of larvated Ascaridia galli eggs, and (2) to study inhibitory effect of X-rays (post larvation) on the development of male A. galli by differentiating between mortality and sex reversal, and by determining the extent and time of occurrence of this effect, (3) to study possible X-ray induced effects on the survival, growth rate, morphology and antigenicity of worms.

REVIEW OF LITERATURE

Effects of Irradiation on Helminth Eggs

Thomas and Quastler (1950) reported no effects when "dry cultures" containing eggs of Rhabditis strongyloides were exposed to 100, 400, 1,600, 6,400 and 10,000 roentgens (r) respectively. Similar "wet cultures" were irradiated with 10,000, 20,000 and 40,000r, respectively. Exposure to 10,000r produced no effect when compared with controls. The intestine of R. strongyloides is normally dark but many worms when irradiated with 20,000r showed light areas in their intestines. No adults were found in

the 40,000r culture after normal development time; however, when adult worms eventually developed, they were sterile. Eggs produced by the females were abnormally shaped. Upon X-irradiation of "dry cultures" 40,000r of exposure produced the same effect as 20,000r exposure of "wet cultures".

Babero (1952) exposed groups of unembryonated Ascaridia galli eggs to X-rays at dosages of 5,000, 10,000, 20,000 and 40,000 roentgens. He observed that death among the lower dosages (5,000 and 10,000r) occurred just before or after gastrulation whereas, greatest percentage of deaths among the higher dosages (20,000 and 40,000r) occurred before the blastula stage. He reported abnormalities in larvae developing from X-irradiated eggs. (e.g. esophageal swellings, hypertrophied cells of the reproductive organs, and light and dark areas in the intestine). No size difference was reported between larvae of the same age developing from irradiated and control eggs.

Bachofer and Pahl (1955) and Pahl and Bachofer (1957) reported that X-irradiation delayed cleavage of Ascaris suum eggs and interfered with normal embryogenesis. They found maximum survival if the post irradiation temperature was approximately that of maximum biological activity. If post-irradiated eggs were held at 0°C and 5°C for periods up to 35 weeks before incubation at 30°C there was no change in the cleavage delay but there was a decrease in survival. Pahl and Bachofer (1957) found the time required for cleavage of irradiated eggs reduced by an anaerobic treatment after irradiation. This treatment also increased the percentage of eggs which completed embryogenesis. Eggs required aerobic incubation after anaerobic treatment. Incubation for 15 hours or more at 30°C immediately after irradiation gave maximum recovery of eggs. Similar results were obtained by a post irradiation treatment with cyanide.

Bachofer (1957) exposed Ascaris suum eggs to X-rays and ultra-violet radiation. X-rays produced greater mortality (70%) and increased cleavage time (50%) while a dose of ultraviolet radiation, producing the same increase in promuclear fission time, resulted in 5% mortality and a 65% increase in cleavage time.

Shikhobalova, et al. (1958a) exposed eggs of Ascaris lumbricoides and A. suum to X-rays or gamma rays from Co^{60} prior to cleavage. Sixty to 100% of the eggs exposed to 2,000-15,000r (X-ray) and 5 to 26% of those exposed to 30,000r developed whereas, 40,000r was lethal. Development of larvae after all irradiations was retarded. Shikhobalova, et al. (1958b) in a later experiment used eggs from the same two species as well as Ascaridia galli. Eggs X-irradiated at various stages of development, i.e. zygote to infective larvae, showed that the eggs were most sensitive to irradiation at the blastula, morula, and early gastrula stages. Criteria of sensitivity were the number of infective larvae developing and their ability to migrate within the host. Ability to migrate was affected more when eggs were X-irradiated at the zygote stage than after larvation was completed. They reported an increase in the percentage of females in irradiated groups.

Villella et al. (1958) irradiated Ascaris suum eggs with gamma rays from Co^{60}. Dosages of 30,000 and 100,000 rep (roentgen equivalent physical)* retarded larval development. Embryonated eggs were more susceptible to irradiation than unsegmented eggs. An exposure of infected eggs to 100,000-150,000 rep was necessary to prevent development of viable larvae in the lungs of Guinea pigs. Some degree of pneumonitis occurred after a dose as

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* The dose of any ionizing radiation which produces the energy absorption of 93 ergs/gm. in tissue.

high as 250,000 rep. An X-ray dose of 100,000r was effective in preventing larval development in lungs of Guinea pigs.

Bubnow (1959) irradiated eggs of Ascaris and Trichuris from pigs and Fasciola eggs with 84,600 - 1,621,880r from Co^{60}. Eggs irradiated in feces failed to develop in contrast to eggs in untreated feces. Similar experiments, using X-rays at 2,000-5,000r revealed that development of Trichuris muris eggs was similar to that of untreated eggs. At a dose of 10,000r development was delayed and 1.7% of the embryos reached the invasive stage whereas 100,000r produced 100% mortality (Shikhabalova and Paruzkinskaya 1960). X-irradiation of T. muris eggs after larvation with 20,000 and 40,000r completely stopped post-embryonic development. Dosages of 5,000 and 10,000r delayed development of larvae and reduced their invasive ability. The same doses had no action on eggs at the stage of zygote formation (Shikhobalova and Paruzkinskaya, 1961). Kumagai (1961), using gamma rays from Co^{60} at dosages of 10^4, 10^5, $1.3x10^5$, $2.0x10^5$, $4.0x10^5$ $6.9x10^5$ and $1.35x10^6$r found that the great majority of Ascaris eggs exposed died when doses exceeded $5.0x10^5$r. Delay in development increased with increasing amounts of irradiation. Soeno (1961) reported that maintaining a constant total of $1x10^5$r of gamma rays from Co^{60} while increasing dose rates ($3.2x10^4$, $8.8x10^5$ and $6.4x10^5$r/hr.) resulted in a corresponding increase in cleavage time of Ascaris eggs. This delay was manifested by the fourth day after irradiation.

Cysts of the golden nematode, Heterodera rostochiensis, were exposed to doses of 20, 40 and 80 Kr of X-ray respectively. Increased mortality resulted with increasing dosages. Irradiation of larvae with the same levels resulted in a decrease in the percentage of fertile eggs in females developing from these larvae (16, 2 and 0% for the 20, 40 and 80 kr groups

respectively). Results indicated that the presence of sperm may be necessary for release and maturation of oocytes, Fassuliotis (1961).

Shikhobalova and Paruzkinskaya (1962) found that T. muris eggs were most susceptible to X-irradiation at the eighth blastomere and morula stages as few eggs exposed to 5,000r at these stages developed into mature worms in mice. No infective eggs developed after exposure to 10,000r and those infective larvae which developed from eggs exposed to 10,000r or 20,000r at the blastula stage did not mature in mice.

Syngamus skrjabinomorpha eggs were found to be highly sensitive to X-rays at 5,000r or more during the 4-8th blastomere stage. Development was retarded and deaths occurred in later embryonic stages or during larval development in infected chicks. Twenty-one days after infection, chicks given 400 eggs per os contained an average of 4.9 pairs of worms which developed from eggs irradiated with a dosage of 5,000r as compared with 40.6 pairs in chicks given untreated eggs. Few larvae from eggs exposed to 5,000r in the infective stage matured in chicks although eggs in the same stage of development were largely resistant to a dose of 2,000r of X-rays (Shikhobalova and Paruzkinskaya, 1962).

Effects of Irradiation on Helminth Larvae

Gould et al. (1953) exposed in vitro cultures of Trichinella spiralis larvae to 750,000r of filtered X-ray and found almost complete mortality in 1-2 hours. Exposure with 3,500r largely prevented reproduction while 5,000-6,000r also halted maturation. A dose of 15,000r given 1.45 cm thick pieces of trichinous meat rendered it non-infective to rats.

Larvae of Trichinella exposed to 10,000r of gamma rays (Co^{60}) and then given to rats demonstrated a markedly reduced pathogenicity when compared with a non-irradiated control group. All rats (12) in the control died while

all those (12) in the irradiated group lived. Most adult worms recovered were sterile (Gould et al., 1955).

Gould et al. (1956) irradiated T. spiralis larvae with 1,000 to 4,000r of X-ray and with 1,000-15,000r of Co^{60}, which were fed later to rate and recovered 3-6 days later. They found, as did Gould et al. (1957), that higher doses produced a decrease in body length of worms. Six days after exposure to 2,000r of X-ray only a few females examined were found to be inseminated. Increased dosages resulted in decreased insemination and increased degeneration of egg cells.

Gordon et al. (1960) exposed infective larvae of Trichostrongylus colubriformis to 20,000, 40,000, 60,000 and 100,000r of X-ray respectively. Guinea pigs were infected with 5,000 to 100,000 larvae and later sacrificed. As X-ray dose increased there was a decrease in the total number of worms recovered and in the percentage of males. Jennings et al. (1963) found that inactivation of Nippostrongylus brasiliensis depended on the total dose of radiation administered and was independent of the rate of delivery within a range of 235-735r/min.

Miller (1964) found that X-irradiation of the infective larvae of Ancylostoma caninum reduced the infectivity of larvae as measured by subsequent intestinal establishment of adult hookworms. As radiation dosage increased there was a corresponding decrease in infectivity by larvae resulting in a reduction of the worm burden accompanied by a decreased pathogenicity to the host. Male larvae were more sensitive to X-ray than female larvae, especially at higher levels. All female worms developing from larvae exposed to 40 Kr and higher were sterile.

Effects of Irradiation on the Antigenicity of Helminths

Levin et al. (1942) vaccinated rats with 4,000 Trichinella spiralis

larvae which had been given 3,250-3,750r of X-ray. Rats were challenged at various time intervals with 2,500 or 10,000 normal larvae. X-irradiated larvae grew to maturity in the intestine but produced no offspring (no muscle invasion). An immunity to the challenge dose was produced. It was therefore concluded that the origin of a mechanism of host resistance to Trichinella is located in the intestine.

When rats were fed Trichinella spiralis larvae which had been exposed to 10,000r Co^{60} (results in sterilization of worms but does not inhibit their development) a definite degree of immunity to re-infection developed. Few adults developed if 18,000r were given as opposed to 10,000r; however, little or no immunity resulted (Gould et al., 1955).

Dogs given 1,000 Uncinaria stenocephala larvae exposed to 40,000r of X-ray and later challenged with normal larvae showed a strong immunity, (Dow et al., 1958). In a field trial Jarrett et al., (1958) vaccinated calves with 1,000 X-irradiated (40,000r) Dictyocalus viviparious larvae, and on challenge with 4,000 normal larvae, found no immunity.

Jarrett et al., (1958) exposed Haemonchus contortus larvae to 10,000, 20,000, 40,000, 60,000, and 100,000r, respectively. Vaccination of sheep with larvae from the 40,000 and 60,000r groups produced less immunity, indicating that larvae irradiated at this level failed to survive long enough in the host to serve an antigenic function. Ross et al. (1959) vaccinated calves with 10,000 X-irradiated Haemonchus larvae (60,000r) and found only a 60% reduction in the number of worms. The X-ray dose was believed to be excessive to that required for immunity.

Larsh et al. (1959) showed that mice given 5 vaccinations with X-irradiated T. spiralis larvae (7,000r) developed about the same degree of immunity as those given the same number of previous infections with

non-irradiated larvae. The titer of serum antibodies was considerably higher in the latter case. Gordon et al. (1960) reported that Guinea pigs given irradiated or normal Trichostrongylus colubriformis larvae and later challenged (20,000r larvae) showed a strong immunity.

Dictyocaulus filaria larvae exposed to 40,000r of X-ray were used as a vaccine for lambs. Two doses of 1,000 and 2,000 larvae, respectively were given 30 days apart. Upon challenge with 4,000 or 80,000 normal larvae an 81.1% immunity (based on number of worms recovered) was found among vaccinated lambs when compared with a control group receiving no vaccination (Sokolic et al., 1963). Miller (1964) found that a single subcutaneous vaccination of dogs (3 months old) with 1,000 X-irradiated Ancylostoma caninum larvae (40,000r) conferred a highly significant resistance.

The following is a summary of knowledge on the effects of irradiation on helminths to date. As work on the effects of irradiation on helminths progressed it became evident that exposure to increased roentgen doses was followed by a corresponding increase in the mortality of eggs and worms. The magnitude of this effect was found to vary directly with progressive stages of development, e.g. embryonated eggs were more susceptible to irradiation than unsegmented eggs. It was noted that irradiation of unsegmented eggs resulted in retardment of cleavage based on the time required to complete embryogenesis. A decrease in the infectivity of worms (based on number recovered upon subsequent infection of the host) occurred with increasing roentgen doses. Recovered worms often exhibited a retardation of growth as well as certain abnormalities such as light and dark intestinal areas and abnormal eggs. Several workers report a reduction in the ratio of males to females although this effect was lacking in some species. An

increased antigenicity has been shown for larvae of some species upon attenuation by irradiation.

MATERIALS AND METHODS

Adult Ascaridia galli were obtained from chicken intestines collected at poultry produce firms in Manhattan and Salina, Kansas. Intestines were flushed using the hydraulic method of Ackert and Nolf (1929) and worms were recovered by straining through a 10 mesh sieve. Worms for Experiment 1a were refrigerated in 0.5% formalin (7°C). Because this technique resulted in failure of many eggs to adhere to dishes, worms obtained for all other experiments were refrigerated in a solution of 1 drop of 1:1000 merthiolate* per 20 cc of 0.85 saline (Larson, 1957). Cultures of Ascaridia eggs were prepared by Larson's (1957) modification of the technique of Hansen et al. (1954) and were refrigerated (7°C) until 14 or 15 days prior to X-irradiation. Several worms were used to prepare cultures in Petri dishes and only a single culture was used in an experiment since Shikhobolova and Paruzkinskaya (1961b) reported a considerable variation in viability of eggs of A. galli from different females. Each culture dish in Experiment 1a was filled to a depth of $\frac{1}{4}$ inch with a merthiolate solution; 1 drop 1:1000 merthiolate per 30 cc of distilled water. This culture medium was found to be unsatisfactory because it favored the growth of bacteria which were able to destroy A. galli eggs. A solution of 0.2% formalin was found to be effective in controlling these bacteria, therefore, this medium was used in experiments 1b through 3d. Approximately 2 weeks prior to irradiation, dishes were placed in an

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* Eli Lilly, Indianapolis, Indiana

incubator (30°C) and egg cultures allowed to larvate. Cultures were sometimes combined in order to have a sufficient number of eggs on each plate. A small amount of culture fluid was added to eggs which were then loosened from contributing dishes (containing few eggs) and poured into higher count dishes. Due to extensive clumping and floating of larvated eggs in the formalin culture medium, this technique was used only during preparation of plates for Experiments 1b through 3d inclusively and not after refrigeration or during incubation.

Examination of eggs was made with a Compound Research microscope and adapter (Ostlind and Hansen, 1964). During examination enough culture liquid was kept in the dish to keep eggs moist. Dishes were examined at various times during incubation to determine the extent of bacterial growth and the number of eggs larvating. The total number of eggs was calculated from the number of eggs counted in a small known area. The total area of the 90 mm diameter Petri dish was 63.3 sq cm. Width of the low power field (100x) on a B & L Compound microscope using 10x oculars was 0.202 cm as determined with a stage micrometer. Total length of the observable field was 7.2 cm because the objective would not cover 0.9 cm on either end due to its striking the side of the dish. Total area observed across the diameter therefore was 1.5 sq cm. (0.202x7.2). Dividing 1.5 sq cm. into total area of dish (63.6 sq cm.) gave a multiplication factor of 42.4. For the 90x10 mm dish using 10x oculars on a triocular B & L compound microscope the multiplication factor was 85.7.

In Experiments 2c and 2d, six 50x15 mm dishes of eggs were placed in the incubator allowed to larvate. Eggs were scraped from the outer portion of the dish and discarded. The entire area of remaining eggs was examined and the total number of eggs counted. This process was repeated until

approximately 100 and 150 larvated eggs remained on each dish for Experiments 2c and 2d respectively. Dishes were then returned to the incubator until X-irradiation.

A Picker 150 KV X-ray unit (Serial No. 296) at Kansas State University Physics department was used for all irradiations. X-ray unit operating data were 100 KV and 4 ma without external filter in Experiment 1a. All subsequent experiments (1b through 3d) were performed with the unit operating at 90 KV and 4 ma without external filter. The amount of back scatter irradiation was not determined but was assumed to be the same for all experiments. Distances between X-ray source and eggs varied according to the type of Petri dish used and are shown in Table 1. Eggs were irradiated on a special block (Ostlind and Hansen, 1965). Construction of the block permitted the insertion of a Victoreen r-meter thimble such that its center would be in the same plane as the eggs in the Petri dishes. Calibration measurements (r/2 sec or r/1½ sec.) were made and then averaged to give an average radiation rate (Table 2). The radiation rate thus determined was the amount of X-irradiation to be received by the eggs per unit time with the exception of Experiments 3a, 3b and 3c. In these Experiments distance from source to eggs was 8.1 cm. The X-irradiation rate received by eggs was calculated from the equation: $\frac{r_1^2}{r_2^2} = \frac{I_2}{I_1}$ where $\frac{r_1^2}{r_2^2}$ is the ratio of the square of the distance from source to object and I the intensity of X-rays received at the specified distance. To determine an X-ray rate variation after prolonged use of the unit, calibrations were made before and after 2 hours of operation. Calibration prior to operation gave readings of 16.7, 16.7, 16.7 and 61.4r/1½ sec., a dose rate of 665.0 ± 2.5 r/min (2.5 = a.d. = $\frac{\Sigma|\text{deviation of each reading from mean}|}{n}$). After operation, readings of 16.7, 16.4 and 16.6 r/1½ sec. were obtained, a dose rate of 662.7± 4.5 r/min.

Table 1. Distances from X-ray source to eggs.

Exp. No.	Type dish	Diameter of dish (cm)	Distance (cm)
1a	Kimax	9.0x2.1	14.0
1b	Pyrex	9.0x1.5	13.4
1c	Kimax	9.0x1.2	13.1
2a	Kimax	9.0x1.2	13.1
2b	Pyrex	5.0x1.6	8.1
2c	Pyrex	5.0x1.6	8.1
2d	Pyrex	5.0x1.6	8.1
2e	Pyrex	5.0x1.6	8.1
2f	Pyrex	5.0x1.6	8.1
3a	Pyrex	5.0x1.6	8.1
3b	Pyrex	5.0x1.6	8.1
3c	Pyrex	5.0x1.6	8.1
3d	Pyrex	5.0x1.6	8.1

Table 2. X-irradiation data for all experiments relative to larvated eggs of Ascaridia galli.

Exper. No.	Calibration r/2 sec	Calibration r/1½ sec	Dosage rate (r/min)	Total dose (r)	Error (r)	Exposure (min)	Temperature (°C) In	Temperature (°C) Out
1a	8.6 8.5 8.4 8.2		252.9 ± 3.9*	5,000 10,000 20,000 40,000 80,000	76.8 153.7 306.9 614.2 1,228.5	19.7 39.4 78.7 157.5 315.0	29.5 29.0 29.0 29.0 29.0	27.0 27.0 26.5 27.0 26.5
1b	8.3 8.4 8.3 8.3		249.6 ± 1.0	10,000	42.1	40.1	25.0	23.5
1c	8.2 8.2 8.1		246.1 ± 1.1	10,000	44.7	40.6	23.5	23.0
2a	7.7 7.8 7.8 7.7		232.5 ± 1.5	10,000	64.5	43.0	24.0	23.0
2b		15.5 15.3 15.2	613.2 ± 4.4	10,000 10,000 10,000	71.7 71.7 71.7	16.3 16.3 16.3	25.0 25.0 25.0	23.5 23.0 23.0
2c		16.2 16.2 16.2	648.0 ± 0.0	10,000	-	15.4	27.0	25.5

* a.d. = $\frac{\text{deviation of each reading from the mean}}{n}$

Table 2. Continued.

Exper. No.	Calibration r/2 sec	Calibration r/1½ sec	Dosage rate (r/min)	Total dose (r)	Error (r)	Exposure (min)	Temperature (°C) In	Temperature (°C) Out
		17.3		10,000 (2d)	560.5	14.8	27.0	23.5
2d &		16.6	678.0 ± 38.0	10,000 (2d)	560.5	14.8	26.0	24.5
2e		16.5		10,000 (2d)	560.0	14.8	25.5	24.5
		17.4		10,000 (2e)	560.5	14.8	25.0	24.5
		16.6		10,000	123.5	15.3	24.5	23.5
2f		16.3	652.0 ± 8.0	10,000	123.5	15.3	24.0	23.0
		16.0		10,000	123.5	15.3	23.5	23.0
	8.2							
3a	8.2		651.6 ± 3.2	80,000	392.9	122.8	23.0	23.0
	8.1							
	7.7							
3b &	7.8		615.6 ± 2.9	89,750 (3b)	428.2	145.6	24.0	23.5
3c	7.8			89,750 (3c)			24.0	23.5
	7.7							
		16.3						
3d		16.4	658.0 ± 16.0	80,000	1,945.6	121.6	25.0	24.0
		16.6						
		16.5						

Prior to irradiation of eggs culture fluid depth was adjusted to 1 mm and the temperature of the fluid recorded. The block and X-ray cone were encased in a lead box which was then closed to reduce stray radiation. The unit was turned on and kept running for the required length of time by placing a heavy weight on the foor switch. After exposure time had elapsed, the dish was removed from beneath the cone and temperature of fluid taken immediately. The culture fluid was discarded and fresh fluid added in Experiments 1b thru 3d. In Experiment 1a the exposed medium was changed the next morning. The same procedure was followed for all dishes that required X-irradiation, varying only time of exposure. After all dishes had received their respective dosages they were returned to the laboratory and placed in the incubator. Exposure data are shown in Table 2.

Unsexed chicks for Experiments 1b thru 2a, 3b and 3c were obtained when 2 days old from the De Forest Hatchery, Peabody, Kansas. Those for Experiments 1a, 2c, and 3d were obtained as day-old unsexed chicks from the Manhattan Hatchery, Manhattan, Kansas. Upon arrival all chicks were vaccinated for Newcastle disease* and placed in heated brooders. The day previous to infection birds were weighed and banded. Birds were divided into desired number of groups by the method of Gardiner and Wehr (1950).

Breed of chicks and age at infection are shown in Table 3. Infection of birds in Experiments 1(a thru c), 2c and 3(a thru d) was carried out in the following manner. Petri dishes containing control and irradiated eggs were taken from the incubator and the culture fluid discarded. Plates were washed twice with distilled water to remove any traces of culture fluid. Five ml and 3 ml of 1.25M sugar solution were added to the 9.0 and 5.0 cm

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* American Cyanamid, New York, New York

Table 3. Experimental chicks.

Exp. No.	Breed	Age at infection (days)	Age at challange (days)
1a	Cornish Rock	4	-
1b	White Rock	7	-
1c	White Rock	7	-
2a	White Rock	21	-
2c	White Leghorn	28	-
3a	White Leghorn	7	37
3b	White Leghorn	21	52
3c	White Leghorn	14	45
3d	White Leghorn	6	23

diameter dishes, respectively. The eggs were scraped off the bottom with a rubber policeman and the suspended eggs were poured into 15 ml sputum bottles which had been coated with Desicote. A known volume of the suspension was placed on a slide by means of a calibrated pipet and a cover slip was added. Total number of larvated eggs on the slide was counted. In Experiment 1a each bird was given the volume of suspension necessary to contain the desired number of larvated ova without dilution. This volume was calculated from the equation:

$$\frac{\text{No. larvated eggs}}{\text{Volume examined}} = \frac{\text{Desired No. of larvated eggs}}{X}$$

where X = volume containing desired number of larvated eggs.

In Experiments 1b, 1c, 2e and 3(a thru d) the number of larvated eggs in a known volume was counted by the method used in Experiment 1a. The suspension was then diluted to give the desired number of larvated eggs in a given volume. The dilution required was calculated from the equation:

$$\text{Volume present} \left[\frac{(\text{No. larvated eggs counted})\ (\text{Volume of dose desired})}{(\text{Volume examined})\ (\text{No. larvated eggs desired in dose})} - 1\right] = X$$

where X is the volume to be added. This addition was done by means of a calibrated 1 ml pipet. Volumes and dosages for Experiment 1a are shown in Table 4 while those for Experiments 1b, 1c, 2e and 3(a thru d) are shown in Table 5.

Birds in Experiment 1b were injected with .625 mg. of Hydrocortone*, an immuno-suppressive agent, in order to recover more worms for examination. This was injected into the breast muscle as a 1:3 dilution with .85% saline (Johnson, 1962). Injections were made at 0, 3, 6, 9, 13 and 16 days post infection. Birds in Experiment 1c were injected in a similar manner at 9

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* Merck Sharp and Dohme, Hydrocortone Tertiary Butylacetate

Table 4. Infection of chickens with larvated Ascaridia galli ova, Experiment 1a.

Group	Ova suspension vol. examined (ml)	Larvated ova (no.)	Amount suspension given each bird (ml)	Infective dose
Control	0.075	26	0.43	150±15
5,000r	0.100	28	0.36	100±10
10,000r	0.100	55	0.36	200±20
20,000r	0.100	43	0.34	150±15
40,000r	0.100	75	0.27	200±20
80,000r	0.075	31	0.36	150±15

Table 5. Infection of chickens with larvated Ascaridia galli ova, Experiments 1b, 1c, 2e and 3(a-d).

Exp. No.	Group	Ova suspension vol. examined (ml)	Larvated ova (no.)	Volume dilution (ml)	Amount suspension given each bird (ml)	Infective dose
1b	Control	.1	218	.17	.1	200±20
	10,000r	.1	228	.62	.1	200±20
1c	Control	.1	33	0	.3	100±10
	10,000r	.1	50	0	.2	100±10
2e	Control	.05	132	19.89	.1	30±3
	10,000r	.05	20	1.05	.1	30±3
3a	Control	.1	151	.02	.1	150±15
	80,000r	.1	65	.41	.2	100±10
3b	Control	.1	56	.36	.2	100±10
	89,750r	.1	39	.05	.3	100±10
3c	Control	.1	56	.36	.2	100±10
	89,750r	.1	45	.90	.3	100±10
3d	80,000r	.1	240	1.02	.6	1,000±100

and 12 days post infection. In Experiment 2c (birds were infected with both normal and control eggs) injections of Hydrocortone were given at 0, 3, 6, 9, 12, and 15 days post infection.

In Experiment 2a larvated eggs were hatched by the method of Hansen et al. (1956). Hatched larvae were centrifuged for concentration and diluted with 15 cc Lock's Physiological saline (.85%) in a 45 cc sputum bottle. Solution containing larvae (1.3 cc) was injected into the duodenal loop by means of a syringe and 18-gauge needle. This volume (1.3 cc) was selected on the basis of the original count of total eggs on the plate (prior to hatching) and was such that it would contain the hatched larvae from 100±10 larvated eggs. After injection the sputum bottles were placed in an incubator (45°C) to stimulate the larvae. Volumes of 1.3 cc of control and irradiated groups were placed in 5.0 cm diameter Petri dishes, respectively, and diluted with warm Lock's saline to cover the bottom of the dishes. Guide lines, slightly less than the width of the visable field, were drawn on the bottom of the dish. The dish was examined under a B & L Stereozoom dissecting microscope at 30x magnification. In this manner the entire plate was examined and larvae present were divided into three groups on the basis of motility. These were:

a. Active - Larvae moved with an undulating swimming motion.

b. Inactive - Very slow movements of a portion of the body but no undulating motion.

c. Dead - No movement was observed.

Larvae were returned to the incubator between examinations to prevent cooling.

Larvated eggs in Experiments 2b and 2c were hatched in a similar manner, however larvae were not given to birds. To remove any error resulting from the loss of eggs during removal and transfer of eggs from the plate, a known

volume of eggs was examined after egg shell disolving but prior to hatching (Experiment 2b). From this the total number of larvated eggs was determined. A final volume to be examined after hatching was then selected on the basis of this total number such that it would contain larvae hatched from 100±10 larvated ova. Experiment 2c was designed to determine the accuracy of methods used in Experiments 2a and 2b. All eggs except a known number were removed from the bottom of the plate. Larvae were hatched and final examination was made to determine the per cent recovered.

In Experiment 2d, 6 week old Cornish Rock cockrels were sacrificed and a portion of intestine removed from immediately proximal to the gizzard to approximately 5 inches posterior of the bile duct - intestinal junction. The contents of this portion of intestine were pressed into a beaker. These contents were centrifuged at 1,000 R.P.M. for 5 minutes. The supernatant was poured off and filtered thru No. 2 Whitman filter paper in a Buchner funnel. The sediment was washed with a small amount of 0.85% saline and then discarded. The filtrate and sediment wash were combined and refiltered thru 2 thicknesses of No. 2 Whitman filter paper and then placed in a 40°C incubator until use. Petri dishes were removed from the 30°C incubator and the culture media discarded. Ten ml of the intestinal filtrate were added to completely cover eggs. This was then slightly diluted (5:1) with 0.85% saline. Dishes were then placed in the 40°C incubator for 24 hours. At the end of this time liquid from the dishes was discarded and plates were washed to remove sediment clinging to eggs. Plates were then examined and the number of larvated eggs which had failed to hatch was determined.

Birds in Experiments 3(a thru c) were divided into seven groups designated as follows:

Group 1. Control, vaccinated.

Group 2. Control, vaccinated, no challenge.

Group 3. Control, vaccinated, challenge.

Group 4. Control, non-vaccinated, challenge.

Group 5. X-irradiated, vaccinated.

Group 6. X-irradiated, vaccinated, no challenge.

Group 7. X-irradiated, vaccinated, challenge.

Chicks were vaccinated as shown in Table 5. Birds were challenged with 100±10 normal eggs at 30, 21 and 31 days post vaccination for Experiments 3a, 3b and 3c, respectively. In Experiment 3d only Groups 4, 6 and 7 described above were used. Groups 6 and 7 were vaccinated with 1,000±100 X-irradiated eggs (80,000r). A challenge infection of 100±10 normal larvate eggs was administered 17 days post vaccination.

Birds were killed at various days post infection as shown in Table 6. The number following the dosage in each experiment is the number of groups infected. The number of birds sacrificed on a given day corresponds to the kill day given in the "Days post infection at necropsy" column. For example, in Experiment 1c for the Control dosage, one group was infected. At 7, 20 and 35 days post infection, 3, 3 and 4 birds, respectively, were sacrificed.

Worms were recovered in the following manner, with noted modifications, because of the age of birds at various necropsy dates. In Experiment 1a the small intestine from the gizzard to the cecal junction was removed from each bird and placed in a finger bowl containing 0.85% saline and the wing band. The intestine was cut open and its contents emptied into the bowl. The intestine was put in a 2 oz. jar approximately half-full of 0.85% saline and shaken. The entire contents of the jar were poured into the finger bowl. The intestine was removed and placed in 25 cc of digestion

Table 6. Necropsy days

Exp. No.	Dosage (r)	Days post infection at necropsy	No. birds* sacrificed
1a	0 (Control)(1)**	3, 12, 20, 28, 35	3, 3, 3, 3, 2
	5,000 (1)	3, 12, 20, 28, 35	3, 3, 3, 3, 2
	10,000 (1)	3, 12, 20, 28, 35	3, 3, 3, 3, 2
	20,000 (1)	3, 12, 20, 28, 35	3, 3, 3, 3, 2
	40,000 (1)	3, 12, 20, 28, 35	3, 3, 3, 3, 2
	80,000 (1)	3, 12, 20, 28, 35	3, 3, 3, 3, 2
1b	0 (Control)(1)	8, 10, 12,16,20,65	3, 3, 3,3,3,6
	10,000 (1)	8, 10, 12,16,20,65	3, 3, 3,3,3,6
1c	0 (Control)(1)	7, 20, 35	3, 3, 4
	10,000 (1)	7, 20, 35	3, 3, 4
2a	0 (Control)(1)	23	6
	10,000 (1)	23	6
2e	***	35	6
3a	0 (Control)(4)	30, 63	3, 9
	90,000 (3)	31, 65	3, 6
3b	0 (Control)(4)	31, 65	3, 9
	89,750 (3)	31, 65	3, 6
3c	0 (Control)(4)	31, 65	3, 9
	89,750 (3)	31, 65	3, 6
3d	0 (Control)(1)	37	6
	80,000 (2)	37	3, 6

* Number of birds killed on succeeding kill days, respectively.
** Number of groups in dose.
*** Both X-irradiated and control ova were given to birds.

solution (Herlick, 1956). Each jar was labeled and placed in an over (45°C) for at least 2 hours.

Modifications of the described necropsy technique for the different kills were as follows. Because no intestinal larvae were found on the 4th day of an earlier experiment, the yolk sac, gizzard, liver, crop, ceca and proventriculus were examined on the third day of Experiment 1a. For the 28 and 35 day kills intestines were flushed, to avoid cutting worms and then handled as previously described.

The intestinal contents and digested intestines were examined under low power of a dissecting microscope. Worms were recovered and fixed in 62x16mm vials containing Wards Cestode fixative and the wing band. The glycerol content was increased over a period of time by adding glycerine dropwise (2.5 - 5%) until each vial contained 35% glycerine. Worms were then mounted in lactophenol on 1x3 inch glass slides.

In Experiments 1b and 1c the intestine was removed as previously described. The contents were flushed into a quart jar by the method of Ackert and Nolf, (1929) and filled with water. This was allowed to settle for 15 minutes and excess water was removed by means of an aspirator. For necropsy days 16, 20 and 65 and necropsy days 20 and 35 for Experiments 1b and 1c, respectively, the contents were poured into a black photographic developing pan and examined under bright light. Worms were removed and placed in 62x16mm vials containing Wards Cestode fixative. For the 8, 10 and 20 day kill in Experiment 1b and the 7 day kill in Experiment 1c the flushings were placed in pilsner glasses. The intestine was slit and shaken vigorously with water in a 2 oz. jar. As no worms were found in the digested intestine in Experiment 1a, no digestion was carried out in

experiments. The intestine was rinsed with water and discarded. The rinse water and contents of the jar were added to the pilsner glass and allowed to settle. Excess water was removed by means of an aspirator and the sediment was placed in 2 oz. jars. Formaldehyde was added as necessary to make the liquid in the jar 10% formalin. Bottles were capped, labeled and stored at room temperature until examinations could be made. These were made with low power (7X) of a dissecting microscope. Worms were recovered and placed in 10% formalin. Worms for the 10 and 12 day kill in Experiment 1b were later placed in 70% isopropal alcohol containing 5% glycerine. The alcohol was allowed to evaporate and worms were mounted in glycerine jelly on 1x3 inch glass slides.

In all subsequent experiments worms were recovered by the method described for the 16, 20 and 65 day kills in Experiment 1b. Worms recovered in Experiments 2a and 2e were preserved in Wards cestode fixative. Those recovered in Experiments (3a-d) were preserved in 10% formalin.

Worm lengths were determined in Experiment 1a by two methods. The image of small immature worms was projected on white paper by means of a microprojector then traced. A magnification factor was determined by projecting a stage micrometer at the same distance on the paper. Large worms were traced directly onto onion skin paper. This was done by placing the worms between a light source and ground glass plate which picked up the image. The traced length of each worm was measured by a Dietzgen map measure. Worms to be measured in Experiment 3d were handled in a similar manner. Worms were microscopically examined for morphological variations and comparisons were made between different radiated groups and controls. Measurements of esophagus, tail and spicule length (Experiment 1a) were made with the use of an ocular micrometer and compound microscope.

Morphological measurements were made only on mature worms with maturity based on the presence of eggs or sperm in worms.

Determination of the sex of all worms 10 days old or older was made in Experiments 1(a-c). Male worms 16-65 days old were sexed on the basis of the morphology of the tail (shape of tail and presence of preanal sucker, Plate 1A). Female worms of the same age were sexed on the basis of the shape of the tail and the absence of the preanal sucker (Plate 1B). The determination of sex of 10 and 12 day worms in Experiment 1b was based on the presence or absence of the preanal swelling (note arrow Plate 1C and 1D). The preanal swelling forecasts the preanal sucker of the male which will be present following the third molt, (Ackert, 1931). In a few cases of very young worms it was necessary to base sex on the presence or absence of the vulva (see lower curvature Plate 1F). Worms younger than 10 days showed no reliable characteristics which could be used to determine sex. Examination of eggs from each female Ascaridia (in Experiment 1e) was made by cutting worms in the area of the vulva. A portion of the uterus was pressed out and eggs teased from this. Eggs were placed on a slide and examined to determine fertility, the criteria being the presence of the fertile spot which is characteristic of fertile eggs, (Ackert, 1931).

EXPERIMENTAL RESULTS

Ackert et al. (1931) reported that administering increasing numbers of larvated eggs (50) to White Leghorn chicks (light breed) did not affect the total number of worms developing. In order to determine if a similar relationship existed for the heavy breeds of chickens used in Experiment 1(a-c), a study was made of the egg dosages versus number of worms recovered (Table 7). The results were that increased egg dosages did not result

EXPLANATION OF PLATE I

A. Tail of male Ascaridia galli, 30 days old.

B. Tail of female Ascaridia galli, 30 days old.

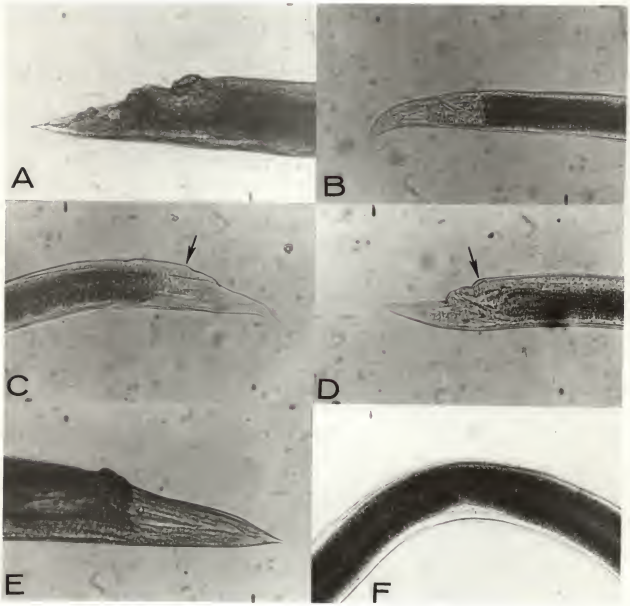
C. Tail of male Ascaridia galli, 10 days old. Arrow designating preanal swelling.

D. Tail of male Ascaridia galli, 12 days old. Arrow designating preanal swelling.

E. Tail of female Ascaridia galli, 12 days old.

F. Vulva of female Ascaridia galli, 12 days old.

PLATE I



in greater worm burdens, thus comparisons between groups and between experiments is valid. Variation between the groups (Table 7) was believed to be due to fluctuations in hatching rate of eggs and to variations in the rate and vigor of peristaltic movements of the fowls' intestines.

The effects of different amounts of X-irradiation of larvated eggs and ultimate survival of worms in the host is shown in Table 8 and Figure 1. A decrease in viability and/or infectivity occurred at the 5,000r level. The mean differences in numbers of worms recovered between the controls and 5,000r and between the 5,000 and 10,000r groups (3.78 and 6.00, respectively) were significant ($LSD_{(.05)}=2.87$). Likewise, larvated ova exposed to 20,000, 40,000 and 80,000r, respectively, resulted in significant mean differences of 5.92, 5.88 and 5.85, respectively, in the number of worms recovered when compared with the control group.

Different X-ray dosages had little effect on the length of 12-day-old worms in Experiment 1a (Table 9 and Figure 2). During the next 8 days (20 days post infection) the 5,000r (2.28 cm), 10,000r (1.76 cm) and 20,000r (1.33 cm) groups grew significantly faster than did the controls (0.69 cm). Worms from the 40,000r (0.71 cm) and 80,000r (0.31 cm) groups showed no significant differences in growth at this date when compared with the Controls (0.69 cm). By the 35th day post infection, worms in the Control (5.44 cm) attained a size comparable to those in the 5,000r (5.04 cm), 10,000r (5.09 cm) and 20,000r (5.25 cm) groups. The length (3.00 cm) of the single immature worm recovered at that time from the 40,000r group was considerably less than the average length of the control worms. No distinction was made in the present study between the lengths of male and female worms prior to 35 days as Ackert (1931) reported little difference

Table 7. Relationship of egg dosage (Ascaridia galli) to worm burden (21 days post infection).

No. larvated ova given	Trial 1			Trial 2			Total		
	Total worms	Avg./ bird	Range	Total worms	Avg./ bird	Range	Total	Avg./	Range
100+10	282	11.3	0-58	327	13.1	0-31	609	12.2	0-58
150+15	187	7.5	0-33	227	9.1	0-37	414	8.3	0-37
200+20	239	9.6	0-44	331	13.2	0-77	670	11.4	0-77

Table 8. Worms recovered in Experiment 1a.

Dosage (r)	No. of birds	Days after infection to necropsy	Total worms recovered	Range	Avg./ bird
0 (Control)	3	3	0	0	0
	3	12	17	2-10	5.7
	3	20	9	2-4	3.0
	3	28	26	7-10	8.7
	2	35	18	8-11	9.0
5,000	3	3	0	0	0
	3	12	11	2-5	3.7
	3	20	7	1-4	2.3
	3	28	4	0-3	1.3
	3	35	12	2-6	4.0
10,000	3	3	0	0	0
	3	12	42	9-22	14.0
	3	20	19	3-12	6.3
	3	28	24	4-15	8.0
	3	35	21	4-13	7.0
20,000	3	3	0	0	0
	3	12	2	0-1	0.7
	3	20	3	1-1	1.0
	3	28	1	0-1	0.3
	3	35	2	0-1	0.3
40,000	3	3	0	0	0
	3	12	3	1-1	1-0
	3	20	4	0-3	1.3
	3	28	1	0-1	0.3
	4	35	1	0-1	0.3
80,000	3	3	0	0	0
	3	12	6	0-4	2.0
	3	20	1	0-1	0.3
	3	28	2	0-2	0.7
	3	35	0	0	0

EXPLANATION OF FIGURE 1

Number of *Ascaridia galli* recovered on various necropsy days, Experiment 1a.

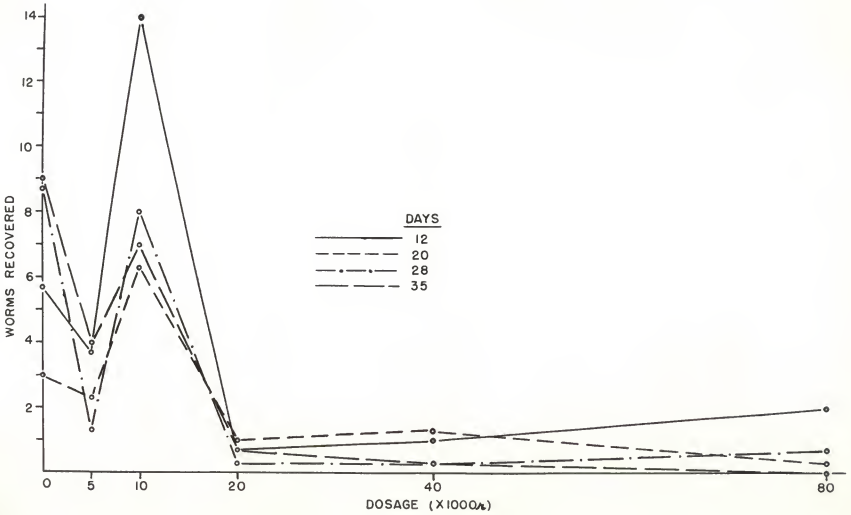
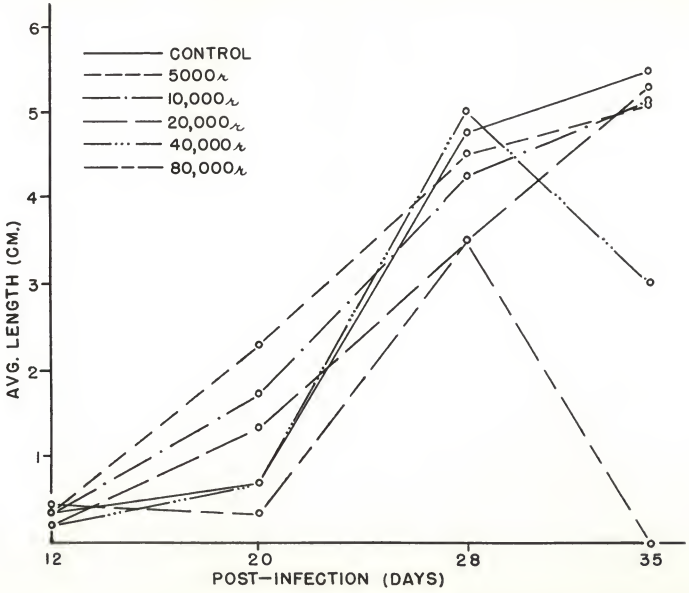

WORMS RECOVERED
14
12
10
8
6
4
2
0
5
10
20
40
80
DOSAGE (X1000λ)
DAYS
12
20
28
35

Table 9. Effects of various dosages of X-ray on growth of Ascaridia galli, Experiment 1a.

Dosage	Days after infection to necropsy	Worms measured (no.)	Range in length (cm)	Average length (cm)
0 (Control)	3	0	0	0
	12	14	0.18-0.49	0.35
	20	8	0.20-1.30	0.69
	28	26	1.50-6.00	4.75
	35	18	4.50-7.00	5.44
5,000	3	0	0	0
	12	3	0.33-0.43	0.38
	20	4	1.50-3.00	2.28
	28	4	3.00-6.00	4.50
	35	12	3.50-5.50	5.04
10,000	3	0	0	0
	12	38	0.10-0.60	0.36
	20	11	0.50-3.50	1.76
	28	24	3.50-5.50	4.27
	35	21	4.50-6.00	5.09
20,000	3	0	0	0
	12	2	0.17-0.25	0.21
	20	3	1.00-2.00	1.33
	28	1	-	3.50
	35	2	4.50-6.00	5.25
40,000	3	0	0	0
	12	3	0.15-0.31	0.21
	20	3	0.24-1.50	0.71
	28	1	-	5.00
	35	1	-	3.00
80,000	3	0	0	0
	12	5	0.14-0.60	0.43
	20	1	-	0.37
	28	2	3.00-4.00	3.50
	35	0	0	0

EXPLANATION OF FIGURE 2

Growth of *Ascaridia galli* originating from eggs exposed to different dosages of X-irradiation, Experiment 1a.


CONTROL
5000r
10,000r
20,000r
40,000r
80,000r
AVG. LENGTH (CM.)
6
5
4
3
2
1
12
20
28
35
POST-INFECTION (DAYS)

in the growth rate of males and females at 19 days of age.

Measurements of the esophagus and tail of mature female worms in Experiment 1a showed that X-ray dosages of various levels had no apparent effect on the development of these structures. The average esophageal length of female worms was 2.93, 2.79 and 2.70 cm for the Control, 5,000r and 10,000r respectively. The average tail length of females was 1.02, 0.99 and 1.01 cm for the Control, 5,000r and 10,000r, respectively.

A comparison of the sex of mature worms recovered in Experiment 1a (Table 10) showed that X-irradiation of larvated ova with 5,000 or 10,000r of X-ray completely inhibited the subsequent development of mature male worms (Tables 10, 11). Number and sex of worms recovered are shown in Table 12 and Figure 3 and in Table 13 and Figure 4 for Experiments 1b and 1c, respectively. In the three Experiments (1a, 1b and 1c) all worms recovered (1,111) from birds infected with larvated eggs exposed to 10,000r of X-ray were females. In Experiment 1b total numbers of worms recivered were 816, 962 and 816 for Control male worms, Control female worms and 10,000r worms (females), respectively. Similarly 181 males (Control), 176 females (Control) and 191 females (10,000r) were recovered in Experiment 1c. In Experiments 1b and 1c, when an immunosuppresser was used, the total number of females recovered from the 10,000r group was 1,007. The total number of Control females recovered in Experiment 1b and 1c was 1,138, the number of Control males 997.

Upon morphological examinations of worms in Experiment 1a dark intestinal areas were found in some nematodes of all groups and an anterior esophageal swelling was present in all worms. Abnormalities were found which were not present in the controls although some were infrequent in

Table 10. Numbers of males and females recovered at 35 days in Experiment 1a.

Dosage (r)	Total	Immature	Mature	
			Males	Females
0 (Control)	18*	1	6	10
5,000	12	1	0	11
10,000	21*	1	0	19
20,000	2	2	0	0
40,000	1	1	0	0
80,000	0	0	0	0

* Tails of cut or broken worms were sometimes missing. Anterior sections were included in the number of worms recovered but not in the number of sexed worms.

Table 11. Number and sex of worms recovered at various necropsy days in Experiment 1a, Control and 10,000r Groups.

Days post infection	Dosage (r)	No. worms* recovered	Range	Avg. No. worms/bird	Total Males	Total Females	Avg. No. males	Avg. No. females	Range males	Range females
12	0 (Control)	17		5.67	7	9	2.33	3.00		
	10,000	42		14.00	0	42	0	14.00		
20	0 (Control)	9		3.00	4	5	1.33	1.67		
	10,000	19		6.33	0	19	0	19.00		
28	0 (Control)	26		8.67	10	14	3.33	4.67		
	10,000	24		8.00	0	24	0	8.00		
35	0 (Control)	18		6.00	6	10	2.00	3.33		
	10,000	21		7.00	0	19	0	7.00		

* Tails of cut or broken worms were sometimes missing. Anterior sections were included in the number of worms recovered but not in the number of sexed worms.

Table 12. Number and sex of worms recovered in Experiment 1b, birds infected with 200 ± 20 larvated ova.

Days post infection	Group Dosage (r)	No. worms recovered	Range	Avg. No. worms	Total males	Total females	Avg. males	Avg. females	Range males	Range females
8	0 (Control)				-	-	-	-	-	-
	10,000r				-	-	-	-	-	-
10	0 (Control)	258	63-111	88.00	108	137	36.00	46.33	21-47	34-56
	10,000r	189	47-73	63.00	0	189	0	63.00	0	47-73
12	0 (Control)	524*	82-337*	131.00	239	276	59.75	69.00	38-152	42-177
	10,000r	215	59-83	61.67	0	215	0	61.67	0	59-83
16	0 (Control)	348	97-149	116.00	139	200	43.66	66.67	40-52	54-90
	10,000r	97	31-36	32.33	0	97	0	32.33	0	31-36
20	0 (Control)	460	105-212	153.33	225	231	75.00	77.00	47-102	57-107
	10,000r	174	33-84	58.00	0	174	0	58.00	0	33-84
65	0 (Control)	225	17-61	37.50	105	118	17.50	19.67	7-28	10.37
	10,000r	141	6-57	23.50	0	141	0	23.50	0	6-57

* One bird received 400 40 ova.

EXPLANATION OF FIGURE 3

Effects of X-irradiation on sex of *Ascaridia galli*, Experiment 1b.

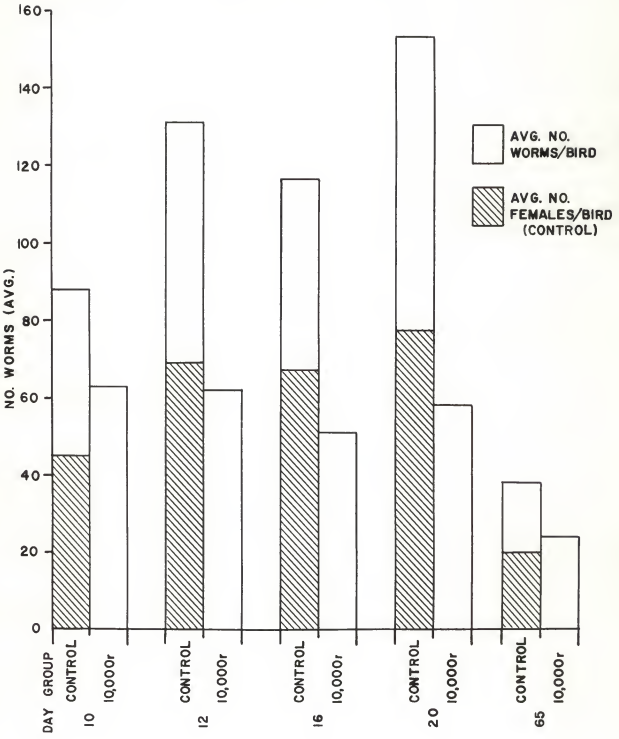
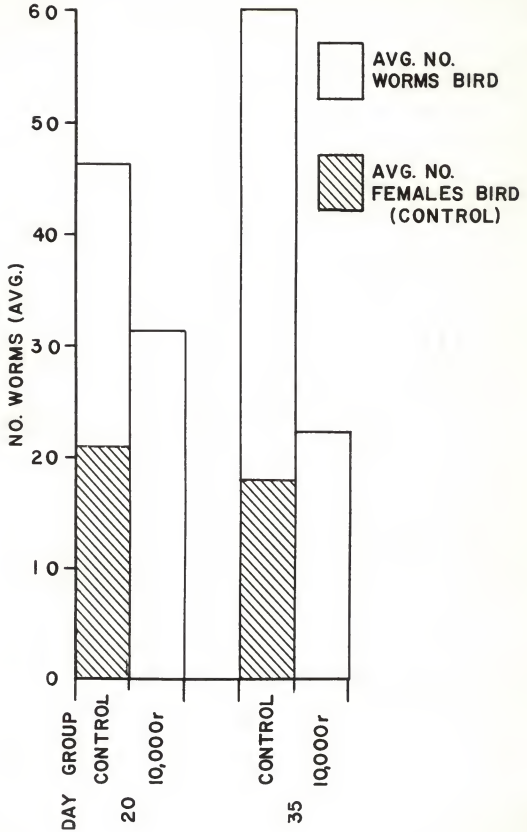

NO. WORMS (AVG.)
0
20
40
60
80
100
120
140
160
AVG. NO. WORMS/BIRD
AVG. NO. FEMALES/BIRD (CONTROL)
DAY GROUP
CONTROL
10,000r
10
12
16
20
65

Table 13. Number and sex of worms recovered in Experiment 1c.

Days post infection	Dosage (r)	Total worms	Range	Avg. No. worms	Total males	Total females	Avg. males	Avg. females	Range males	Range females
20	0 (Control)	139	30-62	46.33	54	63	18.00	21.00	16-20	14-42
	10,000r	94	24-37	31.33	0	94	0	31.33	0	24-37
35	0 (Control)	240	52-76	60.00	127	113	31.75	28.25	27-43	25-33
	10,000r	97	29-35	32.33	0	97	0	32.33	0	29-35

EXPLANATION OF FIGURE 4

Effects of X-irradiation on sex of Ascaridia galli, Experiment 1c.


60
50
40
30
20
10
0
NO. WORMS (AVG.)
AVG. NO. WORMS BIRD
AVG. NO. FEMALES BIRD (CONTROL)
DAY GROUP
CONTROL
10,000r
CONTROL
10,000r
20
35

occurrence. Abnormalities found in Experiment 1a were a shortened posterior uterine branch and a coiling of the intestine. In Experiment 1b, two of the mature females (65 days post infection) from the 10,000r group showed a marked suppression in the development of the reproductive system. Only a short branch of the vagina on either side of the vulva developed. Female reproductive organs among 10,000r worms were found to range in development from the condition previously described to complete development. Approximately 20% of the females exhibited a markedly reduced uterus. All eggs examined from the uteri of female Ascaridia galli which developed from eggs exposed to 10,000r of X-ray after larvation were abnormally shaped (Plate 2B-F). The two outer layers of the shell, as described by Ackert (1931), were lacking and the vitelline membrane was often blistered (see Plate 2D).

The results of Experiment 2a, in which artificial hatching of eggs was carried out by the method of Hansen et al. (1956), are shown in Table 14. The average number of active larvae found in 1.3 cc of the solution was 89 and 45 for the control and 10,000r plates respectively. In Experiment 2b an average of 76 active larvae was observed for the two control plates. The average number of active larvae in the 10,000r plates was 95 (Table 15). In Experiment 1c (designed to determine the accuracy of the above recoveries) all except 197 and 161 larvated ova were removed from the control and 10,000r plate, respectively. After hatching 31 larvae were recovered from the control plate (17.3%) and 23 from the 10,000r (14.3%) plate. Results of hatching larvae by the duodenal loop contents method (Experiment 2d) are shown in Table 16. The average per cent of eggs hatched (51.78%) in the 10,000r group was not significantly different when compared

EXPLANATION OF PLATE 2

A. Normal egg from Ascaridia galli (control female).

B.-F. Abnormal eggs from Ascaridia galli (10,000r females).

PLATE 2

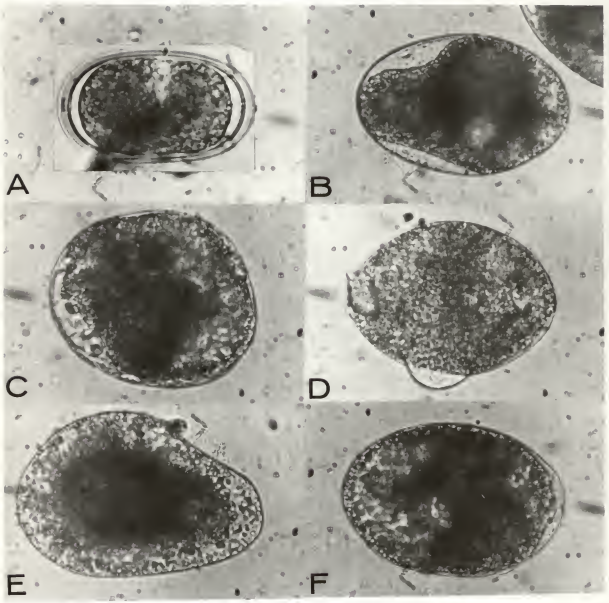


Table 14. Number of larvae in 1.3 cc of solution, Experiment 2a.

Dosage	Count 1			Count 2		
	Active	Inactive	Dead	Active	Inactive	Dead
0 (Control)	86	1	4	92	1	4
10,000r	36	5	10	54	6	6

Table 15. Number of larvae recovered from artificial hatching, Experiment 2b.

Dosage	Plate 1			Plate 2		
	Active	Inactive	Dead	Active	Inactive	Dead
0 (Control)	71	15	4	80	13	6
10,000r	102	8	4	83	14	7

to the controls (54.70%). If the duodenal contents of older birds (12 weeks) were used, there was a decrease in the percentage of ova hatching.

The sex and fertility of worms recovered in Experiment 2e are shown in Table 17. No females containing normal eggs were found in birds which lacked mature male worms (birds 3-6). In those cases where birds did contain mature male worms (birds 1 and 2), all but one of the female worms recovered contained normal eggs. Abnormal eggs (lacking shells) were similar in appearance to those previously described for 10,000r females (Plate 2).

In Experiment 3a an average of 6.3 worms were recovered from birds vaccinated with 100±10 larvated Ascaridia galli eggs, X-irradiated with 80,000r post larvation, as compared to an average of 8.7 worms from birds receiving a challenge dosage but no initial infection (Table 18). This difference was not significant. When birds were given an initial infection of normal ova and later challenged, an average of 41.7 worms per bird was recovered. The sum of the average initial infection (Group 2) and the average challenge infection (Group 4) was 36.0.

Results of Experiments 3b and 3c are shown in Tables 19 and 20, respectively. These experiments were designed not only to confirm results from Experiment 3a but also to determine if age of birds at vaccination and challenge had any influence on the effectiveness of the challenge dose. Birds in Experiment 3c received challenge eggs from the same suspension as birds in Experiment 3b. In Experiment 3b no challenge worms were recovered. Birds in Group 4 (Challenge only) in Experiment 3c had an average of 1 worm per bird. When Group 4 (1 worm/bird) was compared to Group 7 (0.6 worm/bird) no immunity was indicated. Forty-one worms were recovered from the dropping pan of Group 3.

In Experiment 3d the average number of worms recovered at necropsy

Table 16. Larvae recovered after artificial hatching, Experiment 2d.

Trial	Group	Larvated eggs before hatching (No.)	Larvaed eggs after hatching (No.)	Eggs hatched (%)
1	Control	146	55	62.33
	10,000r	138	54	60.87
2	Control	107	61	42.99
	10,000r	167	89	46.71
3	Control	211	87	58.77
	10,000r	157	82	47.77
Total	Control	464	203	54.70
	10,000r	462	225	51.78

Table 17. Sex, maturity and fertility of Ascaridia galli recovered, Experiment 2e.

Bird No.	Males		Females		Type of eggs		
	Mature	Immature	Mature	Immature	Normal (Fertile)	Normal (Infertile)	Abnormal
1	4	0	11	1	8	2	1
2	2	0	2	0	2	0	0
3	0	0	3	0	0	0	3
4	0	1	4	0	0	0	4
5	0	0	0	0	0	0	0
6	0	1	1	1	0	0	1

Table 18. Worms recovered at necropsy, Experiment 3a, vaccination 150±15 (Control) and 100±10 (X-irradiated) larvated ova, 3 birds/group.

Group	Vaccination (Type of ova)	Challenge (Normal ova)	Total worms	Average	Range
1	Control	100±10	54	18.0	12-27
2	Control	None	82	27.3	2-66
3	Control	100±10	125	41.7	1-122
4	None	100±10	26	8.7	0-18
5	80,000r	None	0	0	0
6	80,000r	None	0	0	0
7	80,000r	100±10	19	6.3	0-14

Table 19. Worms recovered at necropsy, Experiment 3b, vaccination 100±10 (Control) and 100±10 (X-irradiated) larvated ova, 3 birds/group.

Group	Vaccination (Type of ova)	Challenge (Normal ova)	Total worms	Average	Range
1	Control	100±10	4	1.3	0-2
2	Control	None	4	1.3	0-3
3	Control	100±10	2	0.6	0-1
4	None	100±10	0	0	0
5	89,750r	None	0	0	0
6	89,750r	None	0	0	0
7	89,750r	100±10	0	0	0

Table 20. Worms recovered at necropsy, Experiment 3c, vaccination 100±10 (Control) and 100±10 (X-irradiated) larvated ova, 3 birds/group.

Group	Vaccination (Type of ova)	Challenge (Normal ova)	Total worms	Average	Range
1	Control	100±10	17	5.7	1-9
2	Control	None	54	18.0	1-51
3	Control	100±10	19	6.3	5-7
4	None	100±10	3	1.0	0-2
5	89,750r	None	0	0	0
6	89,750r	None	0	0	0
7	89,750r	100±10	2	0.6	0-2

from birds vaccinated with 1,000±100 larvated eggs, exposed to 80,000r of X-ray, was 6.5 (Table 21). This was not significant when compared with the number of worms (4.2) recovered from a control group receiving only a challenge infection. All worms recovered from both groups were assumed to be from the challenge infection as no worms were recovered from birds given only X-irradiated eggs (80,000r, post larvation). Results of Experiments 3a-3d (Figure 5) show a direct correlation between the age of birds at challenge and the number of challenge worms recovered. This indicated that older birds did not serve as effective host in immunity studies due to the failure of challenge infections to survive. In Experiment 3d a total of 14 female and 11 male worms was found in the control group as opposed to 22 female and 16 male worms in the 80,000r group. Average length of worms was 1.13, 1.39, 1.96 and 2.12 cm for control (male), 80,000r (male), control (female) and 80,000r (female), respectively.

DISCUSSION

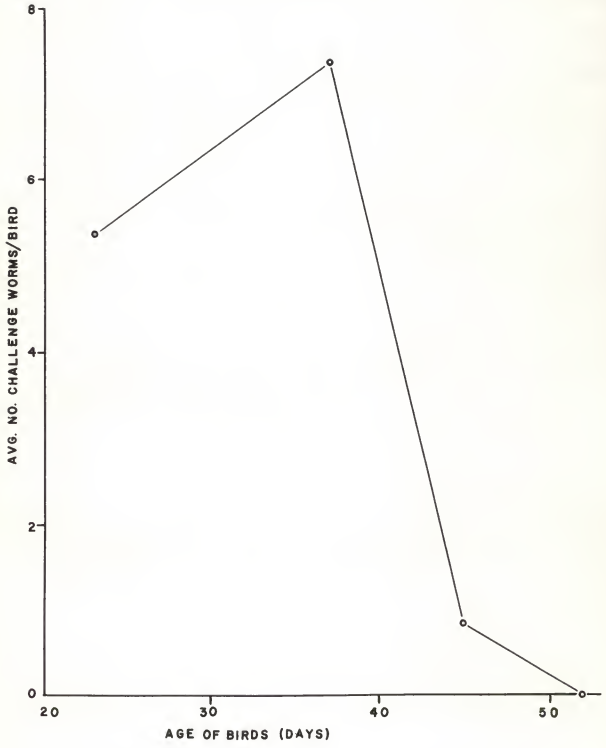
In general, increasing the amount of X-irradiation given larvated Ascaridia galli eggs decreased the number of worms recovered from birds upon subsequent infection. No explanation is given for the decrease in viability and/or infectivity occurring at the 5,000r level in Experiment 1a. At 10,000r the number of worms recovered was comparable to that recovered from the control group. The inverse of this effect (an increase followed by a decrease in infectivity) has been observed on nematodes and other invertebrates by Ciordia and Bizzell (1960), Manwell (1961), Schneider (1960) and Schneider and Porter (1960). An increase in infectivity was also observed with Ascaridia galli which developed from eggs given low dosages of X-ray, pre-larvation, Ruff et al. (1965).

Table 21. Worms recovered at necropsy, Experiment 3d, 6 birds/group.

Group	Vaccination	Challenge	Total worms	Average	Range
Control	None	100±10	25	4.2	2-6
80,000r	1,000±100	100±10	39	6.5	0-28
80,000r	1,000±100	None	0	0	0

EXPLANATION OF FIGURE 5

Average number of challenge worms recovered/ bird from various aged birds.


8
6
4
2
0
AVG. NO. CHALLENGE WORMS/BIRD
20
30
40
50
AGE OF BIRDS (DAYS)

Babero (1952) reported no size difference in Ascaridia galli of the same age which developed from exposed or non-exposed eggs. Results of Experiment 1a indicated that although X-irradiation had no effect on the length of 12-day-old worms, by 20 days post infection a significant difference was noticeable. Higher irradiation dosages (40,000 and 80,000r) significantly retarded growth. Gould et al. (1956) found that higher doses (15,000r of Co^{60} and 4,000r of X-ray) produced a decrease in body length of Trichinella spiralis.

A comparison of sex of Ascaridia galli recovered from birds indicated that exposure to X-rays after larvation of eggs completely inhibited the subsequent development of males. Workers such as Miller (1964) and Gordon et al. (1960) have reported a reduction in the number of males of other nematodes which were recovered from hosts infected with X-irradiated eggs or larvae. The total number of female worms recovered in Experiments 1b and 1c from the 10,000r group was slightly less than the total number of female worms from the control group indicating that 10,000r of X-rays produced some fatalities among female worms although it was not extensive. The fact that only about one half as many worms were recovered from the 10,000r group as compared to the control group indicated that the lack of males was due to their mortality rather than any sex reversal. The total number of worms (females) from the 10,000r group was about what one would expect using an immuno-suppressive agent if only the females were recovered. No morphological evidence of sex reversal was noted in any of the worms examined.

Upon morphological examination of worms in this study, dark intestinal areas were found in some nematodes of all groups. Babero (1952)

reported light and dark intestinal areas present among Ascaridia galli which had received high dosages (20,000 and 40,000r) pre-larvation, but not present in controls and lower dosages (5,000-20,000r). Thomas and Quastler (1950) found that exposure of Rhabditis strongyloides to 20,000r of X-ray resulted in light and dark intestines while exposure to 10,000r produced no such effect. Babero (1952) reported that an anterior esophageal swelling was present in high dosage groups (20,000 and 40,000r) but not in controls and lower dosages (5,000-20,000r). The anterior esophageal swelling was present in all worms including the controls in the present study. This esophageal swelling is characteristic of the rhabditiform esophagus of larvae reported by Ackert (1931).

The results of Experiment 2e (birds fed both X-irradiated and non-irradiated eggs) show a deviation from the expected ratio, found in the preceeding experiments, of 10,000r females to control females. As this ratio was approximately 1:1 if no other factors were involved one would therefore expect approximately half of the females recovered from each bird to contain normal eggs and half to contain the characteristic abnormally shaped eggs of exposed females. It will be noted, however, that in all cases where mature males were absent there was a corresponding lack of females possessing normal shaped eggs, either fertile or infertile. Instead all eggs from these worms were abnormally shaped, possessed a blistered vitellian membrane and all lacked egg shells. On the other hand the presence of mature males was accompanied by a reversal of this situation. With one exception females recovered from birds containing mature males contained eggs which were normal in shape and possessed the typical three layered shell. The eggs from two of the females were

infertile but otherwise completely normal in appearance. One other female contained abnormal eggs. These results suggest that a situation may exist which is homologous to that described in the case of Ascaris lumbricoides in which non-fertilization results in the release of eggs which are different in shape from those from fertilized females. In other words the abnormal eggs found in all females in the 10,000r group may have been the result of non-fertilization due to the lack of males rather than an X-ray induced abnormality. Fassuliotis (1961) believed that the presence of sperm was necessary for the release and maturation of the oocytes of Heterodera rostochiensis. Gould et al. (1956) found that decreased insemination of Trichinella spiralis was accompanied by an increased degeneration of egg shells. Results of hatching larvae by the methods of Hansen et al. (1956) (Experiment 2a) suggested that mortality of male larvae in the 10,000r group occurred in the egg prior to hatching as only approximately half as many larvae were recovered, after hatching, from this group as from the control. Upon a subsequent experiment these results were nonrepeatable suggesting that this method was not sufficiently accurate to determine the time of male mortality. In a carefully controlled experiment where a known number of eggs were hatched recovery percentages were so small as to cast doubt on the reliability of data obtained. It is possible that the many transfers of eggs and/or larvae resulted in many being lost. A new method for the artificial hatching of eggs was therefore developed using the contents of the duodenal loop as previously described. Known numbers of eggs could be directly observed thereby eliminating any error due to their loss in transfer. A much larger percentage of hatching could thus be observed greatly increasing the reliability of data obtained.

The failure of most eggs to hatch when the duodenal loop contents of older birds was used (compared to young birds) has not been explained. Some stimulatory substance and/or a certain physiological state of the contents might be lacking in older birds. It may also be that the source of age resistance in birds is an antibody or another anti-hatching agent in the intestine. This agent would therefore also be effective in an artificial hatching situation. Gould et al. (1955) and Levin et al. (1942) believed the site of resistance to Trichinella spiralis in mice to be in the lumen of the intestine rather than in the muscle or the blood stream.

As no significant difference in the per cent of eggs hatched existed between the control and 10,000r group, results suggest that male larvae are capable of freeing themselves from the egg shell. The lack of any definite sexual characteristics prior to the second molt makes it impossible to determine the sex of larvae recovered prior to 8 days on the basis of morphological characteristics. All worms recovered (3rd and 4th stage larvae and adults) from the 10,000r groups were females. The sum of these results indicates that mortality of male Ascaridia galli, from eggs exposed to 10,000r of X-ray post larvation, occurs sometime after hatching but prior to the third molt and penetration of the intestinal mucosa. The inability to recover sufficient numbers of 1-8-day-old larvae makes a determination of time of death, during this period, on a straight number of worms recovered basis ineffective.

Data from Experiments 3(a-d) indicated that no X-ray induced immunity to subsequent infections resulted from the vaccination of birds with 100±10 or 1,000±100 larvated eggs which had been exposed to 80,000 or more roentgens of X-ray. The immunity which was present seemed to be an age

immunity which resulted in a failure of large numbers of the challenge infection to become established. Decreasing susceptability to infection with increasing age had previously been noted by Ackert et al. (1928). This lack of survival of challenge worms (best seen in Experiment 3b) along with the tendency of older birds to lose the majority of heavy infections (Experiment 3c) makes it imperative that birds used in antigen studies be vaccinated and challenged when as young as possible. Results of Experiment 3d indicated that vaccination did not condition the sex of challenge worms nor was there any retardment of growth rate among challenge worms developing in vaccinated birds. Sokolic et al. (1963) found retardment of growth to be an indication of immunity with Dictyocaulus filaria although no change in the sex ratio was observed.

Several possibilities exist as to why no immunity was found. Those nematodes which have been shown to have an antigenicity effect upon irradiation (Ancylostoma caninum, Dictyocaulus viviparous, Haemonchus contortus and others) all either migrate in their respective host or they have long periods of contact with host tissues during their life cycle. In other words it may be that contact between Ascaridia galli and its host is not of a long enough duration for stimulation of sufficient antibody production. Larsh et al. (1959) found the titer of serum antibodies to be considerably higher in mice infected with X-irradiated larvae. Immunity might be shown if a double vaccination technique were used. Workers such as Jarrett et al. (1959), Jarrett et al. (1961) and Miller (1965) have reported a much higher degree of resistance induced by double vaccination than with a single vaccination with X-irradiated larvae of Dictyocaulus viviparous, Haemonchus contortus and Ancylostoma caninum, respectively.

Larsh et al. (1956), however, found no immunity among mice vaccinated 5 times with X-irradiated Trichinella when compared to controls vaccinated with normal larvae.

The total dosage of X-rays given (80,000-89,750) may have been excessive. Mortality of large numbers of larvae may have occurred before sufficient antibody production was stimulated. Jarrett et al. (1959) found immunity in sheep to be greater after vaccination with Haemonchus larvae X-irradiated with 60,000r than it was with larvae exposed to 100,000r. Ross et al. (1959) believed that even 60,000r was excessive. With Trichinella spiralis vaccination with larvae exposed to 8,000r (inhibition of development of most adults) produced little or no immunity while exposure to 10,000r (development of sterile adults) resulted in a definite degree of immunity, (Gould et al., 1955).

SUMMARY

Larvated Ascaridia galli eggs were exposed to X-ray dosages of 5,000, 10,000, 20,000, 40,000 and 80,000r, respectively and administered to chicks. Several birds of each group were killed and necropsied at 3, 12, 20, 28 and 35 days, respectively. Worms were recovered, measured and examined for morphological abnormalities. No male worms were present in the X-irradiation groups (5,000-80,000r). A significant decrease in viability and/or infectivity occurred in the 5,000r group when compared with the control and 10,000r groups, based on the number of worms recovered. Larvated eggs exposed to 20,000, 40,000 and 80,000r, respectively, resulted in a significant decrease in the number of worms recovered when compared with the controls. A significantly faster growth occurred among worms in the 5,000, 10,000 and 20,000r groups when compared with the controls between 20 and 28

days post infection. There was no significant difference in the average length of esophagus and tail of 10,000r mature female worms when compared with control females of the same age.

Larvated eggs exposed to 10,000r of X-ray were fed to birds. An immunosuppressive agent (Hydrocortone) was given by inter-muscular injection to increase the worm burden. All worms (10 days post infection or older) recovered from these birds were females. The absence of definite sexual morphological characteristics prior to this age prevented the sexing of younger worms (10-day-old). A comparison with the number of females recovered from control birds indicated that mortality rather than a sex reversal of males occurred.

Duodenal loop contents of birds were placed on a known number of larvated control and X-irradiated (10,000r) eggs and the per cent hatch determined. No significant difference in the per cent hatching between groups was found indicating that potential male larvae were capable of freeing themselves from the egg. Thus mortality of male worms occurred after hatching but prior to 10 days post infection (the earliest necropsy date at which worms could be sexed). An increase in the age of birds used for the duodenal contents (from 6 to 12 weeks old) resulted in a corresponding decrease in the per cent hatched.

Morphological examination of worms revealed abnormalities among mature female worms from X-irradiated eggs which were not present in the control females. These included light and dark intestinal areas, a coiling of the intestine, reduced reproductive organs and abnormally shaped eggs which lacked a shell. All eggs from female worms developing from X-irradiated eggs were abnormal. Infection of birds with both control and X-irradiated eggs indicated that the observed abnormality among eggs resulted from not

being fertilized (no males present) rather than from effects of X-irradiation.

Birds were vaccinated with 100±10 or 1,000±100 larvated eggs (exposed to 80,000 or 89,750r). Birds were later challenged with normal eggs and killed (20 days post challenge). A comparison of number of worms recovered, between control and vaccinated birds, indicated no immunity had been conferred by the irradiated larvae.

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EFFECT OF X-IRRADIATION (POST LARVATION) ON DEVELOPMENT, MORTALITY AND ANTIGENICITY OF *Ascaridia galli* (Schrank, 1788)

by

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In recent years there has been a rapid increase in the use of ionizing radiation in many fields of physical and biological science. An increasing amount of research has been performed aimed at the elucidation of the effects of ionizing radiation on biological systems, including parasites and their hosts, especially in the areas of public health and veterinary medicine. Along with such practical and economically important uses of radiation, much basic research must be done to expand knowledge of irradiation effects.

In this study larvated Ascaridia galli eggs were exposed to X-ray dosages of 5,000, 10,000, 20,000, 40,000 and 80,000r and then given to chicks. Several birds of each group were killed and necropsied at 3, 12, 20, 28 and 35 days, respectively. Only female worms were present in the X-irradiation groups (5,000-80,000r). Based upon the number of worms recovered at necropsy, a significant decrease in viability and/or infectivity occurred in the 5,000r group when compared with the control and 10,000r group. Larvated eggs exposed to 20,000, 40,000 and 80,000r, respectively, resulted in a decrease in the number of worms recovered when compared with the controls. From 20 to 28 days post infection worms in the 5,000, 10,000 and 20,000r group were of a significantly greater length than controls. There was no significant difference in the average length of esophagus and tail of 10,000r mature female worms when compared with the control females.

Larvated eggs exposed to 10,000r of X-ray were fed to birds. Injections of Hydrocortone were given as an immunosuppresor in order to increase worm burden. All worms (1,007) recovered 12 days or later from these birds were females. The absence of morphological differences between sexes prior to this time prevented the sexing of younger worms (10-day-old). A comparison with the number of females recovered from control birds (1,138) indicated that mortality among males occurred rather than a sex reversal.

Contents squeezed from the lumen of the duodenal loop of birds were placed on a known number of larvated control and X-irradiated (10,000r) eggs and the per cent hatch determined. No significant difference in the per cent hatching between groups was found indicating that potential male larvae were capable of freeing themselves from the egg after irradiation. Thus mortality of male worms occurred after hatching but prior to 10 days post infection, our earliest necropsy date at which worms could be sexed. Duodenal contents removed from birds older than 6 weeks (12 weeks) were not as effective for hatching eggs as duodenal contents from younger birds.

Morphological examinations of worms revealed abnormalities among mature female worms from X-irradiated eggs which were not present in the control females, (i.e. light and dark intestinal areas, a coiling of the intestine and reduced reproductive organs). All eggs from female worms in the X-irradiated group were abnormally shaped and lacked a shell. Infection of birds with both control and X-irradiated eggs indicated that the observed abnormality among eggs resulted from not being fertilized (no males present) rather than from effects of X-irradiation.

To determine if larvae exposed to X-rays would have an antigenic effect upon that host, birds were given 100±10 or 1,000±100 larvated eggs (exposed to 80,000 or 89,750r). When challenged later with normal eggs and necropsied 20 days post challenge, the number of worms recovered between the control and vaccinated birds indicated no immunity had been conferred by the irradiated larvae.